REVIEW

Diagnostics for Dermatologic Diseases with Autoantibodies

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Background: Dermatologic diseases with autoantibodies were recognized early as autoimmunity became accepted as a pathogenic immunologic concept. Laboratory testing to identify disease-defining autoantibodies and investigate their role in pathophysiology has evolved since.

Content: Blistering dermatologic diseases, profiled by autoantibody production, target epithelial components critical in cell–cell and cell–matrix adhesion, resulting in epithelial separation and other characteristic features of the disorders. This review covers the clinical indications for dermatologic disease-related autoantibody testing, the specifics of procuring specimens to test, the available diagnostic tests, and information provided by the testing. Atypical, uncharacteristic, and less well-known clinical and autoantibody profiles as well as several of the many future prospects for expansion of the testing applications are elaborated on in the online Data Supplement.

Summary: Autoantibody-associated dermatologic diseases are acquired immunologic disorders that have considerable clinical implications affecting essential barrier functions of skin and mucous membranes and causing discomfort, including pain and pruritus. Certain of the diseases can have life-threatening manifestations, and treatments can have significant side-effects. The skin diseases may presage other clinical associations that are important to recognize and treat. Laboratory testing aids in the diagnosis of these diseases through identification of the autoantibodies and is essential for prompt and precise knowledge of the disease type for prognosis, further clinical evaluations, and treatment decisions.

HISTORY AND INTRODUCTION

The first description of autoantibodies in a dermatologic disease was published in 1964 (1). By the late 1960s, the diagnostic utility of immunodermatology testing had been established. This review focuses on the current state of immunodermatology testing, including its applications in diagnosing, defining, and understanding autoimmune cutaneous immunopathology. Clinically applicable information is summarized in Tables 1 and 2 (expanded in Table 1S in the online Data Supplement) and in additional Supplemental Tables and Figures.

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IMPACT STATEMENT

Blistering characterizes dermatologic diseases with autoantibodies targeting components in skin and mucous membranes essential for cell–cell and cell–matrix adhesion. Certain of these diseases are increasing in incidence. The diseases are distinctive but demonstrate clinical overlap. Common presentations such as pruritus, eczema, urticaria, and mucositis, with or without blistering, often are dominant and prolonged, especially in prodromal stages, leading to diagnostic delays. Laboratory tests identifying disease-associated autoantibodies are important for diagnosis and disease monitoring. Prompt and precise diagnosis is needed for treatment decisions, prognosis, and addressing potential associations, including intestinal disorders, other autoimmune diseases, neurologic diseases, drug exposures, and, especially, malignancy.

INDICATIONS FOR TESTING

Clinical immunodermatology tests are diagnostic aids in many diseases that affect skin and mucous membranes. The clinical presentations are varied with overlapping features in diseases with different specific diagnostic markers. Table 1 lists diseases for which testing is indicated. Blistering is a classical, but not exclusive or required, finding in the immunobullous diseases in which autoantibodies target epithelial adhesion components. Disorders included in the differential diagnosis of autoimmune dermatologic diseases should be considered for testing, including common conditions such as eczema, urticaria, pruritus, aphthosis, and mucositis that are, otherwise, of unexplained etiology (Supplemental Table 2). Immunodermatology testing helps to establish an immunopathological diagnostic profile and identify autoantibodies with which to monitor disease expression and activity. The findings are highly sensitive and specific for certain diseases, and levels of autoantibodies determined by the testing often correlate with disease activity. Also, the testing can be valuable in recognizing other potentially associated, significant medical conditions, such as dermatitis herpetiformis (DH) with celiac disease (CD), pemphigus and pemphigoid as manifestations of drug hypersensitivity, and a subtype of pemphigoid with risk for underlying or developing malignancy.

DIAGNOSTIC TESTING FOR AUTOIMMUNE DERMATOLOGIC DISEASES

Advances in autoantibody testing for dermatologic diseases have burgeoned since the original description identifying epithelial intercellular autoantibodies in the sera of patients with pemphigus (1, 3). Direct immunofluorescence (DIF) to localize autoantibodies and markers of their activities in cutaneous tissue remains the mainstay of diagnostic testing. Indirect immunofluorescence (IIF) serum testing provides semiquantitative assessments of the antibody presence and reveals certain categories of diseases based on antibody localization. Autoantibodies in pemphigoid and epidermolysis bullosa acquisita (EBA) each localize on separate sides of split skin substrate, roof, and

Table 1. Dermatologic diseases with autoantibodies.

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
Basement membrane zone (BMZ) antibody-associated diseases		
Pemphigoid		
Bullous pemphigoid (BP) Tense bullae often on urticarial base, Asboe–Hansen sign[c] elicitable, persistent urticarial papules and plaques, flexural distribution, erosions, prominent pruritus with and without blistering, develops in older adults. Clinical variants: infantile/childhood; localized (lower extremities); erythrodermic (generalized erythema); vulvar (prepubertal); vegetating plaque (groin and axillae); herpetiform (vesicles); dyshidrosiform (palmar and plantar resembling dyshidrotic eczema); pemphigoid nodularis (prurigo nodularis-like lesions on extremities); lichen planus pemphigoides (see later), and drug-induced.	hemidesmosome and lamina lucida BP180 (NC16A), BP230	Tissue DIF: Linear, n-serrated[d], BMZ IgG ± C3 or C3 alone Serum IIF: IgG BMZ antibodies, epidermal (roof) or combined epidermal-dermal (roof and floor) with SSS, IgG BMZ antibodies with ME Serum ELISAs: IgG BP180 and/or IgG BP230 antibody levels increased
Mucous membrane pemphigoid (MMP) Cicatricial pemphigoid Ocular cicatricial pemphigoid Brunsting–Perry pemphigoid Anti-laminin-332 pemphigoid (formerly anti-epiligrin pemphigoid and anti-laminin-5 pemphigoid) Ruptured bullae and erosions, rarely intact vesicles, primarily oral and ocular mucous membranes but any mucosa and skin; scarring sequelae. **Antilaminin-332 pemphigoid associated with malignancy** (up to 30%). Brunsting–Perry with scarring head and neck and nonscarring mucosal involvement	hemidesmosome and lamina lucida and lamina densa BP180, laminin-332, laminin-311, BP230, alpha6beta4 integrin (alpha6 epitopes in oral pemphigoid; beta4 epitopes in ocular pemphigoid)	Tissue DIF: Linear, n-serrated[d], BMZ IgG, IgA ± C3 Serum IIF: IgG BMZ antibodies, epidermal (roof) or dermal (floor) with SSS, IgG BMZ antibodies with ME, (IgG laminin-332 antibody assay in validation) Serum ELISAs: IgG BP180 and/or IgG BP230 antibody levels may be increased
Pemphigoid gestationis (PG) (formerly herpes gestationis) Tense bullae, similar to BP, pruritic urticarial papules with onset during or immediately after pregnancy, periumbilical lesions typical; may flare with menses and/or hormonal treatment and recur with subsequent pregnancies.	hemidesmosome and lamina lucida BP180 (NC16A), also rarely BP230	Tissue DIF: Linear BMZ C3 typically intense, ± linear BMZ IgG Serum IIF: Complement-fixing BMZ antibodies, epidermal (roof) with SSS = herpes gestationis factor (HGF); also, IgG BMZ antibodies, epidermal (roof) with SSS and IgG BMZ antibodies with ME in 25% Serum ELISA: IgG BP180 antibody level increased

Continued

Table 1. (continued)

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
p200 (laminin gamma-1) pemphigoid Tense bullae similar to BP, vesicles, and urticarial plaques; also palmoplantar, cephalic, and mucosal involvement; frequent development of scars/milia; possible psoriasis association in approximately 30%.	lamina densa p200 (laminin-311 γ1 subunit)	Tissue DIF: Linear, n-serrated[d], BMZ IgG ± C3 Serum IIF: IgG BMZ antibodies, dermal (floor) with SSS, IgG BMZ antibodies with ME Serum ELISAs: Consider IgG type VII collagen, IgG BP180, and IgG BP230 antibody levels to assess for other or overlapping expression (Serum IgG p200 antibody assay in development)
Epidermolysis bullosa acquisita (EBA) Tense bullae, common in areas of trauma and oral mucosa. Variants: classical/mechanobullous (skin fragility, tense bullae, vesicles, and erosions that heal with scarring and milia); nonclassical/nonmechanobullous (similar features to BP); mucous membrane (predominantly affecting mucosa with squamous epithelia); Brunsting–Perry type (primarily head and neck lesions); IgA presents with linear IgA, instead of IgG (similarity to linear IgA disease but more severe scarring sequelae); inflammatory type (tense bullae on erythematous, urticarial base).	anchoring fibrils type VII collagen	Tissue DIF: Linear, u-serrated[d], BMZ IgG ± C3, IgA, IgM Serum IIF: IgG BMZ antibodies, dermal (floor) with SSS and IgG BMZ antibodies with ME; less common, IgA BMZ, dermal (floor) with SSS and/or IgA BMZ antibodies with ME Serum ELISA: IgG type VII collagen antibody level increased
Linear IgA disease (LAD) (formerly linear IgA bullous dermatosis and chronic bullous disease of childhood) Annular erythema or urticarial plaques with tense bullae and vesicles on skin, described as "necklace-like," "string-of-pearls," "crown of jewels" arrangement, erythema multiforme-like, common oral involvement in adults. Two types based on IgA BMZ localization on split skin substrate, lamina lucida type, epidermal (roof) localization in 91%, and sublamina densa type, dermal (floor) localization in 9%.	hemidesmosome and lamina lucida LAD-1 (LABD97), BP180 (NC16), BP230 lamina densa type VII collagen	Tissue DIF: Linear, n-serrated[d] in common lamina lucida type, and linear, u-serrated[d] in uncommon sublamina densa type, BMZ IgA Serum IIF: IgA BMZ antibodies, epidermal (roof); less common, dermal (floor) or combined epidermal-dermal (roof and floor) with SSS and/or IgA BMZ antibodies with ME
Linear IgA/IgG bullous dermatosis Bullae, erosions, erythematous plaques, oral and genital mucosal involvement	hemidesmosome and lamina lucida and lamina densa BP180, laminin-332, laminin-311, BP230, type VII collagen	Tissue DIF: Linear BMZ IgG, IgA, ± C3 Serum IIF: IgA and IgG BMZ antibodies, common epidermal (roof); less common, dermal (floor) or combined epidermal-dermal (roof and floor) with SSS, IgA and/or IgG BMZ antibodies with ME Serum ELISAs: IgG BP180 and/or IgG BP230 and/or IgG type VII collagen antibody levels increased

Continued

Table 1. (continued)

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
Cell surface (CS)/intercellular substance (ICS), antibody-associated diseases		
Pemphigus		
Pemphigus vulgaris Flaccid bullae on noninflamed skin, crusting, Nikolskiy sign[c] elicitable, Asboe–Hansen sign[c] elicitable, commonly affects oral mucosa, scalp, chest, and intertriginous areas, the fragile bullae rupture and do not remain intact, large, painful erosions are prominent in mucosa and/or skin. Types: mucosal dominant, mucocutaneous, cutaneous	desmosome desmoglein 3 (mucosal dominant), desmoglein 3 with desmoglein 1 (mucocutaneous and cutaneous)	Tissue DIF: Epidermal/epithelial CS/ICS IgG ± C3 or C3 alone Serum IIF[e]: IgG epithelial CS/ICS antibodies; stronger with ME than NS Serum ELISAs[e]: IgG desmoglein 3 antibody level increased with or without lesser IgG desmoglein 1
Pemphigus vegetans Erythematous, vegetating and pustular intertriginous plaques, crusting	desmosome desmoglein 3 with or without desmoglein 1, desmocollin 3	Tissue DIF: Epidermal/epithelial CS/ICS IgG ± C3 or C3 alone Serum IIF[e]: IgG epithelial CS/ICS antibodies; stronger with ME than NS Serum ELISAs[e]: IgG desmoglein 3 antibody level increased with or without lesser IgG desmoglein 1
Pemphigus foliaceus Endemic pemphigus (also known as fogo selvagem) Superficial bullae, erosions, and scale with crusting (fine "corn flake-like" scaling), Nikolskiy sign[c] elicitable. Drug-induced most often this variant.[f] Generalized, erythrodermic presentation also observed. Endemic pemphigus (variant of pemphigus foliaceus with genetic and environmental cofactors, primarily in Brazil; also known as fogo selvagem) superficial erosions, erythema, crusting, localized to seborrheic areas, head, and upper chest	desmosome desmoglein 1	Tissue DIF: Epidermal/epithelial CS/ICS, IgG ± C3 or C3 alone Serum IIF[e]: IgG epithelial CS/ICS antibodies with ME and typically prominent with NS Serum ELISAs[e]: IgG desmoglein 1 antibody level increased and normal IgG desmoglein 3
Pemphigus erythematosus (also known as Senear–Usher syndrome) Variant of pemphigus foliaceus with features of lupus erythematosus. Superficial erosions, erythema, crusting, often of malar and seborrheic areas	desmosome desmoglein 1 and/or desmoglein 3 (antinuclear antibodies also are detected)	Tissue DIF: Epidermal/epithelial CS/ICS, IgG ± C3 or C3 alone and granular immune deposits along BMZ Serum IIF[e]: IgG epithelial CS/ICS antibodies with ME and typically prominent with NS Serum ELISAs[e]: IgG desmoglein 1 antibody level increased and normal IgG desmoglein 3
Pemphigus herpetiformis Erythematous, bullous, vesicular, pustular, or papular lesions, typically with severe pruritus and in a herpetiform pattern; mucosal involvement is uncommon.	desmosome desmoglein 1, less commonly desmoglein 3 (other target antigens have been reported such as desmocollin 1, desmocollin 3178-kDa antigen, BP180 C-terminus, and laminin-332 γ2 subunit)	Tissue DIF: Epidermal/epithelial CS/ICS, IgG ± C3 or C3 alone Serum IIF[e]: IgG epithelial CS/ICS antibodies with ME substrate and typically prominent with NS Serum ELISAs[e]: IgG desmoglein 1 antibody level increased and normal/less commonly increased IgG desmoglein 3

Continued

Table 1. (continued)

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
IgA pemphigus Fragile blisters filled with fluid that evolve into pustules; mucosal involvement is uncommon; pruritus. Variants: subcorneal pustular dermatosis (annular/circinate lesion pattern) and intraepidermal neutrophilic IgA dermatosis (atypical pustules with "sunflower"-like configuration).	desmosome desmocollin 1, desmocollin 2, desmocollin 3, desmoglein 1 and/or desmoglein 3	Tissue DIF: Epidermal/epithelial CS/ICS, IgA Serum IIF: IgA epithelial CS/ICS antibodies with ME and may be prominent with NS
Intercellular IgG/IgA dermatosis Bullous and pustular skin lesions, preferentially on trunk and extremities, also mucosal involvement	desmosome desmoglein 1, desmoglein 3, desmocollin 1, desmocollin 2, desmocollin 3	Tissue DIF: Epidermal/epithelial CS/ICS, IgG and IgA Serum IIF: IgG and IgA epithelial CS/ICS antibodies with ME and NS Serum ELISAs: IgG desmoglein 1 and/or IgG desmoglein 3 antibody levels increased
Cell surface (CS)/intercellular substance (ICS) and basement membrane zone (BMZ) antibody-associated disease		
Paraneoplastic pemphigus (PNP), also referred to as paraneoplastic autoimmune multiorgan syndrome (PAMS) Flaccid and/or tense bullae, erosions, urticarial, lichenoid, or erythema multiforme-like, and flat scaly papules; usually involves oral and ocular mucosa, often extensive including esophageal and respiratory mucosa.	desmosome and hemidesmosome in multiple epithelia (simple, columnar, transitional) desmoglein 3, envoplakin, periplakin, desmoplakin 1, desmoplakin 2, BP230, epiplakin, plectin, BP230, desmoglein 1, desmocollin 1, desmocollin 2, desmocollin 3, alpha-2-macroglobulin-like-1 (A2ML1), laminin-332, and/or BP180	Tissue DIF: Epidermal/epithelial CS/ICS and BMZ, IgG ± C3 Serum IIF: IgG epithelial CS/ICS and BMZ antibodies with rodent (rat and/or mouse) bladder substrate epithelium is characteristic, IgG intercalated discs with rodent heart substrate and IgG portal tracts with rodent liver substrate; also, CS/ICS and BMZ antibodies on ME Serum ELISA: IgG envoplakin antibody level may be increased (in validation)
Miscellaneous-associated diseases		
Dermatitis herpetiformis (also known as Duhring disease) Pruritic, small, symmetrical, grouped vesicles and papules on extensor surfaces (elbows, knees, buttocks); may be replaced by secondary lesions due to consequent scratching; associated with small intestinal gluten-sensitivity/celiac disease (CD).	keratinocyte produced epidermal transglutaminase (eTG/TG3) also tissue transglutaminase (tTG/TG2) in endomysium, principal target of endomysial antibodies (EMA)	Tissue DIF: Granular or fibrillar BMZ ± stippling in dermal papillae, IgA Serum IIF: IgA EMA with distal ME and/or human umbilical cord substrate Serum ELISAs: IgA (tTG/TG2) and IgA (eTG/TG3) antibody levels increased

Continued

Table 1. (continued)

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
Lichen planus Intensely pruritic, papulosquamous, flat-topped, violaceous papular skin lesions, also mucous membrane (oral and genital most common), scalp, and nail involvement with fine white lines, termed "Wickham's striae," on lesion surfaces. Drug-induced presentations and associations with hepatitis B infection and vaccine and hepatitis C-induced liver insufficiency and other autoimmune diseases.Lichenoid reactions may be premalignant and epithelial malignancy associated.	no antibody target; cytoids represent nonspecific staining of dead and dying keratinocytes and/or pieces of BMZ; "shaggy" fibrinogen reflects epidermal-dermal junction interface inflammation	Tissue DIF: Cytoid bodies, few to numerous, IgM common ± IgG, IgA, C3, fibrinogen, and "shaggy" BMZ fibrinogen Serum: No epithelial-directed antibodies
Lichen planus pemphigoides Tense bullae on pruritic violaceous polygonal papules and plaques (lichenoid), "Wickham's striae," on lesion surfaces, mucous membrane (oral and genital most common), and nail involvement.	hemidesmosome and lamina lucida BP180, BP230	Tissue DIF: Linear, n-serrated[d], BMZ, IgG ± C3, cytoid bodies, few to numerous, IgM common ± IgG, IgA, C3, fibrinogen, and "shaggy" BMZ fibrinogen Serum IIF: IgG BMZ antibodies, epidermal (roof) or combined epidermal-dermal (roof and floor) with SSS, IgG BMZ antibodies with ME Serum ELISAs: IgG BP180 and/or IgG BP230 antibody levels increased
Bullous lupus erythematosus Tense bullae, photodistributed, systemic LE ± other cutaneous LE	anchoring fibrils type VII collagen (most common) hemidesmosome and lamina lucida and lamina densa BP180, BP230, laminin-332, laminin-311 uncharacterized epidermal reactivity with or without dermal findings	Tissue DIF: Linear, u-serrated[d], BMZ, IgG ± C3 or C3 alone and granular BMZ Serum IIF: IgG BMZ antibodies, dermal (floor) with SSS; less common, IgA BMZ, dermal (floor) with SSS and/or IgA BMZ antibodies with ME; antinuclear antibodies and other connective tissue disease serologic markers Serum ELISAs: IgG BP180 and/or IgG BP230 antibody levels increased, positive lupus/connective tissue disease serologies; ± abnormal complement levels
Cutaneous lupus erythematosus Malar erythema and swelling, "butterfly rash," photodistributed transient diffuse or papular erythema upper and exposed body and face, spares knuckles on dorsal hands, toxic epidermal necrolysis-like with systemic disease; discoid scaley plaques with follicular prominence ("carpet tack sign"), scarring alopecia and cutaneous scarring, post-inflammatory dyspigmentation; mucositis, cheilitis, nailfold telangiectasias. Drug-induced presentations.	antinuclear antibodies	Tissue DIF: Coarse, granular, continuous BMZ (any or all) IgG, IgM, IgA, C3 ± lichenoid features Serum: Positive lupus/connective tissue disease serologies; ± abnormal complement levels

Continued

Table 1. (continued)

Immunobullous disease Clinical presentation	Target(s) of autoantibodies: Structure(s) Component(s) within structure	Laboratory tests and findings: Tissue direct immunofluorescence[a] (DIF), Serum indirect immunofluorescence[b] (IIF), and Serum ELISA(s)
Subacute cutaneous lupus erythematosus Psoriasiform, papulosquamous or annular, polycyclic plaques with central clearing; dyspigmentation and telangiectasias. Drug-induced presentations.	extractable nuclear antigens, SSA (Ro) and SSB (La)	Tissue DIF: Particulate intercellular IgG, IgM, IgA ± C3 and ± granular BMZ Serum: Positive lupus/connective tissue disease serologies, particularly to extractable nuclear antigen, Ro/SSA
Vasculitis Immune complex, small vessel IgA vasculitis (Henoch–Schönlein purpura) Hypocomplementemic urticarial vasculitis Cryoglobulinemic Petechiae, palpable purpura, nodules, livedo reticularis; resolve with hemosiderin deposition. Systemic involvement including gastrointestinal and renal Associated with chronic infections, autoimmune disease, lymphoproliferative disorders, and drug-induced.	antineutrophil antibodies (uncommon when skin positive); anti-C1q in hypocomplementemic urticarial vasculitis	Tissue DIF: IgM, IgG, and/or IgA, C3, or fibrinogen in and around upper dermal blood vessel walls, and ± granular BMZ; Granular and predominant vascular IgA in IgA vasculitis (Henoch–Schönlein purpura); IgM and IgG vascular predominant and globular in cryoglobulinemic vasculitis Serum: Positive lupus/connective tissue disease/vasculitis serologies, C1q antibodies, and/or cryoglobulins; ± abnormal complement levels

[a] For all suspected immunobullous disease, it is best to obtain biopsy for diagnosis from perilesional tissue because immunoreactants may not be detected by direct immunofluorescence (DIF) in lesional (blistered) tissue; perilesional is defined as immediately adjacent to but not involving a blister or erosion and may include inflamed, intact skin or mucosa. Serum studies may be needed to distinguish diseases.

[b] Substrates for indirect immunofluorescence testing include split skin, also known as salt-split skin (SSS), substrate, monkey esophagus (ME) substrate, intact normal skin (NS) substrate.

[c] The Asboe–Hansen sign is the "bulla spread sign" and refers to the extension of a blister to adjacent, unblistered skin with pressure on top of the blister; it is positive in various blistering diseases. The blister extension, in pemphigoid, has a rounded border and, in pemphigus, has a sharp angle.
The Nikolskiy sign is the formation of a new blister or extension of a blister from shearing pressure applied on normal-appearing skin (direct Nikolskiy sign) or at the edge of an existing blister (marginal Nikolskiy sign). Of note, Nikolskiy often is spelled Nikolsky (without an "i"); however, the more correct translation is Nikolskiy (2).
The Asboe–Hansen sign has been referred to as an "indirect Nikolskiy sign" or as "Nikolskiy II sign.".

[d] See Supplemental Fig. 1 for image examples of serration patterns.

[e] Limiting-dilution, end-point titers by IIF and IgG desmoglein 1 and/or IgG desmoglein 3 antibody levels by ELISAs correlate with disease activity in IgG pemphigus variants.

[f] Drug-induced pemphigus typically resembles pemphigus foliaceus but also possibly pemphigus erythematosus, pemphigus vulgaris, or paraneoplastic pemphigus.

BP230 = bullous pemphigoid antigen 1 (BPAg1); BP180 = bullous pemphigoid antigen 2 (BPAg2), also known as type XVII collagen (COLXVII); cell surface (CS) = intercellular substance (ICS); epidermal refers to the "roof" and dermal refers to the "floor" localization of serum basement membrane zone (BMZ) antibodies on human split skin (also known as salt-split skin) substrate (SSS) by indirect immunofluorescence (IIF).

Abbreviations: **DIF**- direct immunofluorescence, **IIF**—indirect immunofluorescence, **ELISA**—enzyme-linked immunosorbent assay, **BMZ**—basement membrane zone, **CS**—cell surface, **ICS**—intercellular substance, **SSS**—split skin (salt-split skin) substrate, **ME**—monkey esophagus substrate, **NS**—intact normal skin substrate, **BP**—bullous pemphigoid, **EBA**—epidermolysis bullosa acquisita, **LAD**—linear IgA disease, **DH**—dermatitis herpetiformis, **LE**—lupus erythematosus, **PNP**—paraneoplastic pemphigus, **PAMS**—paraneoplastic autoimmune multiorgan syndrome, **EMA**—endomysial antibodies, **eTG**—epidermal transglutaminase, **TG3**—transglutaminase 3, **tTG**—tissue transglutaminase, **TG2**—transglutaminase 2, **NC**—noncollagenous, **HGF**—herpes gestationis factor.

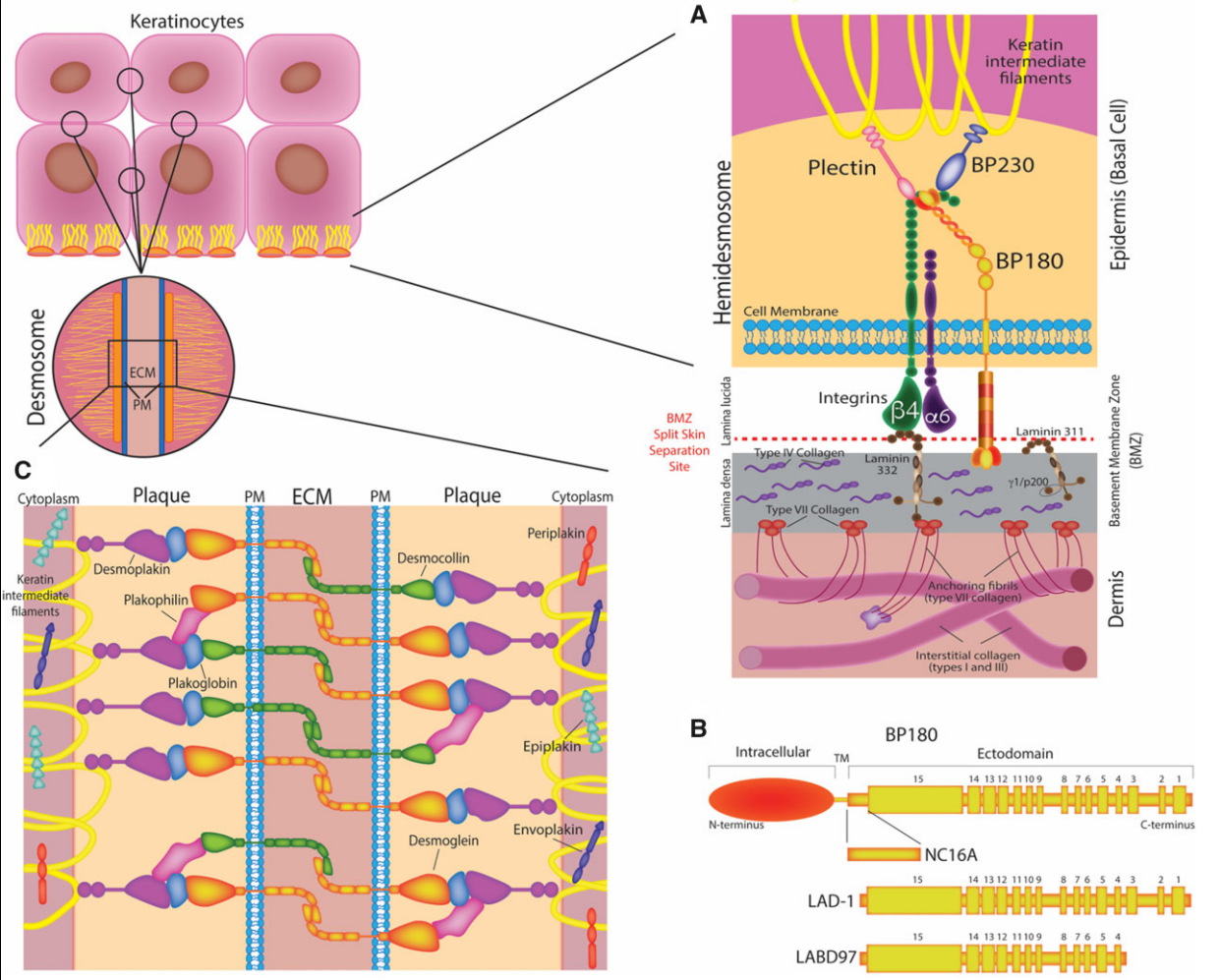

Fig. 1. Schematic representations of epithelial adhesion components and complexes that are targets of autoantibody reactivity in dermatologic diseases. (A) The hemidesmosome **is a specialized multiprotein junctional complex that functions to attach epithelial cells to the underlying basement membrane in stratified and other complex epithelia, including skin, cornea, parts of the gastrointestinal and respiratory tracts, and the amnion. Hemidesmosomes also have signaling functions that critically modulate the organization of the cytoskeleton, proliferation, apoptosis, and differentiation. Autoantibodies in dermatologic diseases with basement membrane zone (BMZ) reactivity are directed to constituents of the hemidesmosomal adhesion complex, schematically represented in the diagram, while mutations in their genes result in inherited bullous disorders often with a similar phenotype (5). Disease-associated targeted hemidesmosomal and BMZ structures and proteins schematically represented in the diagram are recorded in Table 1. Red dotted line indicates where the BMZ split occurs in salt split skin in the lower lamina lucida.** (B) Full-length molecular map of BP180**, also known as bullous pemphigoid antigen 2 (BPAg2) and type XVII collagen (COL XVII), a transmembrane protein with an intracellular domain in the hemidesmosomal dense plaque of basal keratinocytes extending beyond the cell membrane with an ectodomain into the lamina densa of the BMZ. The ectodomain of BP180 contains 15 collagenous domains, labeled by numbers 1 through 15. The noncollagenous extracellular domain of BP180, known as NC16A, is the primary site for antibody binding in bullous pemphigoid and pemphigoid gestationis. IgG serum antibodies are detected via enzyme-linked immunosorbent assay (ELISA) in 80% to 90% of**

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floor, respectively, by IIF and to specific ultrastructurally defined basement membrane zone (BMZ) compartments, the lamina lucida and lamina densa, respectively, by immunoelectron microscopy. Western blotting and immunoprecipitation provide molecular details about the antibody targets (4). This has permitted cloning of targeted structural epithelial components and production of recombinant forms that can readily be used in sensitive assays to provide conclusive evidence of antigen identity by antibodies in multiple specimens, such as in enzyme-linked immunosorbent assays (ELISA). Immunoelectron microscopy, immunoblotting, and immunoprecipitation are useful tools for studies of dermatologic diseases with autoantibodies, but with limited scalability, are generally performed in specialist or research laboratories. ELISA testing is readily available in clinical laboratories, and, together with IIF for sera and DIF for tissues, allows characterization of autoantibodies in dermatologic diseases with sensitivity and specificity; ELISA results alone do not give a complete picture of immunopathophysiology. Figure 1 is a schematic diagram showing the principal adhesion targets of autoantibodies in dermatologic diseases.

Specimens and Tests

Tests are performed on tissue biopsy specimens from skin and mucous membranes as well as serum specimens from patients with suspected diseases. For autoantibody-associated diseases, both tissue and serum testing are most helpful. Biopsy testing initially may give indication for adding serum testing. Serum tests may not be as sensitive as DIF on tissue but are essential to distinguish disease subtypes. Also, serum tests may be positive when tissue tests are not revealing or when a biopsy is difficult to obtain. Moreover, serum testing can be useful in monitoring disease expression because autoantibodies

Fig. 1 (Continued)
affected patients. The production of antibodies to other BP180 epitopes may have clinical significance; for example, antibodies directed against epitopes in the C-terminal end of BP180 have been associated with mucosal disease (6). Fragments of BP180, designated as linear IgA disease antigen 1 (LAD-1), 120 kDa, and linear IgA bullous disease antigen of 97 kDa (LABD97) represent major antigenic targets of autoantibodies in patients with linear IgA disease. These IgA antibodies preferentially react with LAD-1 and LABD97, but not with full-length BP180, indicating that cleavage of the ectodomain BP180 region generates novel autoantigenic epitopes (7). TM**, transmembrane domain;** LAD-1**, linear IgA disease antigen 1;** LABD97**, linear IgA bullous disease antigen of 97 kDa.** (C) The desmosome **is an intracellular and intercellular multiprotein junctional complex that provides mechanical strength and creates strong adhesive bonds between cells. Desmosomes are particularly abundant in tissues subjected to mechanical forces such as epithelium and myocardium. Desmosomes additionally have signaling functions, participating in fundamental processes such as cell proliferation, differentiation, and morphogenesis. Autoantibodies in dermatologic diseases with cell surface reactivity are directed to constituents of the desmosomal adhesion complex, schematically represented in the diagram, that directly disrupt desmosomal adhesion, such that the cells loosen and fall apart (8). Disease-associated targeted desmosomal and intercellular structures and proteins represented in the schematic, mainly desmogleins and desmocollins, are recorded in Table 1. See also Supplemental Fig. 8 with additional information regarding desmoglein and desmocollin epidermal localization. Exfoliative toxins produced by bacterial infections in bullous impetigo and staphylococcal scalded-skin syndrome can cleave desmoglein 1 at a specific site disrupting keratinocyte adhesion, and desmoglein 2 has been identified as a receptor for a subclass of adenoviruses that cause respiratory and urinary tract infections triggering epithelial to mesenchymal cell phenotypic transition. Inherited desmosomal disorders demonstrate broad-ranging phenotypes with either or both cardiac disorders and hair abnormalities. Overexpression of desmosomal proteins occurs in some malignancies in association with tumor progression (9, 10).** PM**, plasma membrane;** ECM**, extra-cellular matrix.**

Table 2. Serum tests for dermatologic diseases with autoantibodies (see expanded version with additional information in Supplemental Table 1).

Indirect immunofluorescence (IIF)						
Disease(s)	bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), other pemphigoid variants, epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), linear IgA/IgG bullous dermatosis	antilaminin-332 pemphigoid	pemphigus foliaceus, pemphigus erythematosus, endemic pemphigus/fogo selvagem,pemphigus vulgaris, pemphigus vegetans, IgA pemphigus, intercellular IgG/IgA dermatosis	paraneoplastic pemphigus (PNP) (also referred to as paraneoplastic autoimmune multiorgan syndrome, PAMS)	dermatitis herpetiformis (DH) and celiac disease (CD)	pemphigoid gestationis (PG)
Autoantibodies	Epithelial basement membrane zone (BMZ) antibodies	Laminin-332 antibodies	Epithelial cell surface (CS), also known as intercellular substance (ICS), antibodies	CS/ICS and BMZ antibodies on rodent substrates	Endomysial antibodies (EMA)	Herpes gestationis factor (HGF)
Immunoglobulin(s)	IgG and/or IgA	IgG	IgG and/or IgA	IgG and/or rarely IgA	IgA and/or rarely IgG	Complement-fixing
Substrate(s)	Monkey esophagus and split skin	Human embryonic kidney cells (HEK)293 cells expressing whole-length recombinant heterotrimeric laminin-332	Monkey esophagus and intact skin	Rat bladder, also mouse bladder, heart, liver	Monkey esophagus and human umbilical cord	Split skin
Enzyme-linked immunosorbent assay (ELISA)						
Disease(s)	bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), pemphigoid gestationis (PG)	epidermolysis bullosa acquisita (EBA) and bullous lupus erythematosus (LE)	pemphigus foliaceus, pemphigus erythematosus, endemic pemphigus/fogo selvagem, pemphigus vulgaris, pemphigus vegetans	paraneoplastic pemphigus (PNP) (also referred to as paraneoplastic autoimmune multiorgan syndrome, PAMS)	dermatitis herpetiformis (DH) and celiac disease (CD)	pemphigoid gestationis (PG)
Autoantibodies	IgG BP180 and IgG BP230 antibodies	IgG type VII collagen antibodies	IgG desmoglein 1 and IgG desmoglein 3 antibodies	IgG envoplakin antibodies	IgA and IgG tissue transglutaminase antibodies and IgA epidermal transglutaminase antibodies	IgG BP180 antibodies

Abbreviations: **DIF**- direct immunofluorescence, **IIF**—indirect immunofluorescence, **ELISA**—enzyme-linked immunosorbent assay, **BMZ**—basement membrane zone, **CS**—cell surface, **ICS**—intercellular substance, **BP**—bullous pemphigoid, **EBA**—epidermolysis bullosa acquisita, **LAD**—linear IgA disease, **DH**—dermatitis herpetiformis, **CD**—celiac disease, **LE**—lupus erythematosus, **PNP**—paraneoplastic pemphigus, **PAMS**—paraneoplastic autoimmune multiorgan syndrome, **EMA**—endomysial antibodies, **eTG**—epidermal transglutaminase, **TG3**—transglutaminase 3, **tTG**—tissue transglutaminase, **TG2**—transglutaminase 2, **NC**—noncollagenous, **HGF**—herpes gestationis factor.
Other mucosal substrates may be substituted for monkey esophagus substrate.
Split skin substrate, also known as salt-split skin, may be human or primate.

correlate with disease activity. Histopathologic examination of a formalin-fixed biopsy specimen can provide a more complete picture of tissue changes related to immunopathology, supporting, but insufficient for, a diagnosis of autoantibody-associated dermatologic disease.

The most crucial aspect of DIF testing, as well as histopathology, is the collection of the specimen and maintenance of its integrity. The tissue site from which an adequately sized biopsy specimen is procured and the transport medium in which the biopsy is placed for transfer to the laboratory are critical for optimal DIF results (consolidated as a reference guide in Supplemental Table 3). Likewise, serum collection and transport conditions are essential to the quality of results.

Tissue DIF testing consists of a panel of fluorescein-conjugated antibodies to determine localization of the antibody targets in a biopsy specimen. Panels typically consist of testing for immunoglobulin (Ig)G, IgM, and IgA, complement component 3 (C3), and fibrinogen. Additional tissue testing may include antibodies to IgG subclasses to enhance sensitivity, antibodies to another complement cascade derivative, C4d, to determine its localization as a more durable marker of complement activation, antibodies to localize epidermal transglutaminase as found distinctively expressed in DH, and antibodies to eosinophil granule proteins to determine tissue deposition as markers of eosinophil-related inflammation, or other inflammatory markers. Most laboratories performing DIF have validated tissue testing of specimens in Michel's or Zeus' transport medium for up to 7–10 days. DIF testing of formalin-fixed, paraffin-embedded tissue has shown limited success. Biopsy specimens exposed to formalin begin to lose immunoreactants in 2–10 minutes (11). Conclusions from comparative studies are that DIF testing of frozen tissue is superior to fixed tissue for immunopathological diagnoses, although certain markers, including C4d and eosinophil granule proteins, are detectable in fixed tissue (12, 13).

Many currently available serum tests that aid in the diagnosis of autoantibody-associated dermatologic diseases are listed in Table 2 with additional information in Supplemental Table 1. IIF plays an important role in diagnosis by classifying the various immunobullous diseases, based on the pattern of antibody reactivity with various substrates and the immunoglobulin class of the antibody. IIF may support a diagnosis when DIF is nondiagnostic or not feasible to perform. Classification by pattern includes separating disorders with cell surface antibodies (pemphigus) from those with BMZ antibodies (pemphigoid and its variants) and also subclassifying BMZ antibody-associated diseases based on epidermal (roof) or dermal (floor) localization on split skin substrate. Testing for antibodies in different immunoglobulin classes aids in distinguishing IgG-mediated disease from IgA-mediated disease, which typically indicates distinct therapies. Serum testing by IIF also provides semiquantitative assessments of antibody levels based on limiting-dilution, end-point titers. The substrate type is important in IIF testing and typically has been animal or human tissues. Sensitivity and specificity of the substrates vary for the different diseases, and the use of multiple substrates increases sensitivity. Further information about IIF substrates is provided in the online Data Supplement. Newly developed IIF tests with transfected cells expressing recombinant target antigens as substrates link IIF and ELISA methodologies (14).

Serum testing also includes ELISA to identify antibodies reactive with specific target antigens, including BMZ antigens, BP180 and BP230 [bullous pemphigoid (BP)], and type VII collagen [EBA and bullous lupus erythematosus (LE)], and cell surface (CS)/intercellular substance (ICS) antigens, desmogleins 1, and 3 [pemphigus foliaceus (PF) and pemphigus vulgaris (PV)]. ELISA testing is

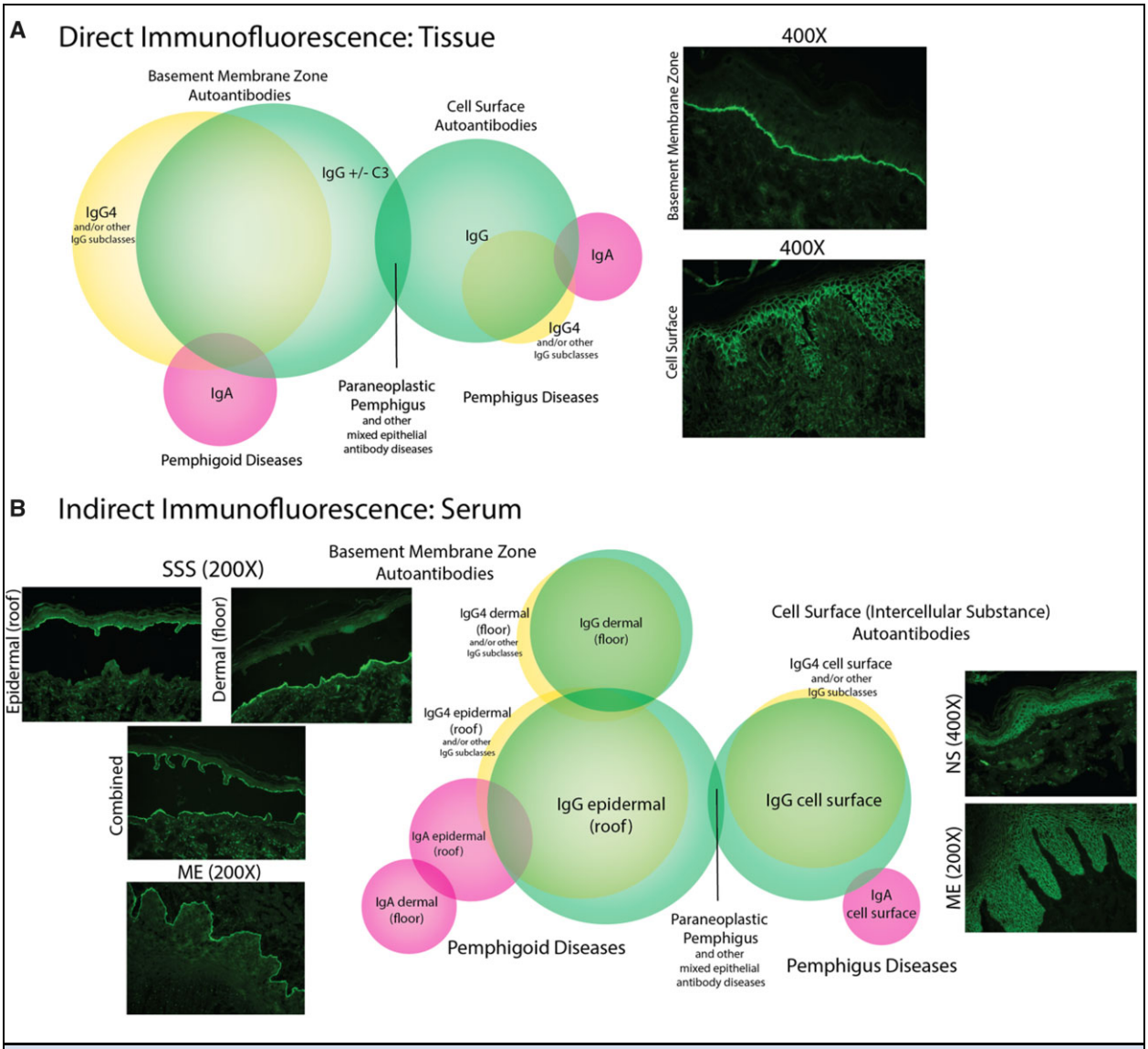

Fig. 2. Venn diagrams representing epithelial basement membrane zone (BMZ) and cell surface (CS)/intercellular substance (ICS) autoantibody reactivity in dermatologic diseases determined by direct immunofluorescence (DIF) on tissue and indirect immunofluorescence (IIF) with serum. The size and overlap of individual circles depict approximate distributions and frequencies of the autoantibody immunoglobulin classes and staining patterns. Representative immunofluorescence images demonstrate characteristic diagnostic test findings (×200 or ×400 denotes original magnification of the photomicrographs). **(A)** In the pemphigoid disorders**, DIF testing on skin or mucosal biopsy specimens demonstrates linear BMZ antibody reactivity, often with a marker of complement activation, third complement component, C3 localization, because of autoantibodies targeting hemidesmosomal and other BMZ antigens. IgG BMZ antibodies, with or without C3 and with or without less intense IgM and IgA BMZ antibody reactivity, are found in pemphigoid and pemphigoid variants, including epidermolysis bullosa acquisita (EBA), and bullous lupus erythematosus (LE). Testing for IgG4 or other IgG subclass reactivity may increase diagnostic sensitivity by DIF. IgA BMZ antibodies predominate in linear IgA disease. Disorders with both IgA and IgG BMZ reactivity, including mucous membrane pemphigoid, IgG/IgA pemphigoid,**

Continued

limited to the specific domain within the assay, such as the noncollagenous (NC) 16A domain of BP180, which is a major antigenic target in patients with BP. ELISA may be more sensitive than IIF and provides value in assessing response to treatment because semiquantitative levels of autoantibodies to epithelial targets by ELISA correlate with disease activity in certain diseases

Fig. 2 (Continued)
other pemphigoid variants, and linear IgA/IgG bullous dermatosis, have implications for disease severity and treatment considerations. (Upper DIF photomicrographic image demonstrates linear BMZ antibody reactivity characteristic of pemphigoid disorders). In the pemphigus disorders, DIF testing **on skin or mucosal biopsy specimens demonstrates epithelial CS/ICS antibody reactivity in a continuous net-like pattern, often with C3 localization, because of autoantibodies targeting desmosomal and other intercellular antigens. IgG CS/ICS antibodies, with or without C3, are found in IgG pemphigus variants, including pemphigus foliaceus and pemphigus vulgaris. Testing for IgG4 or other IgG subclass reactivity may increase diagnostic sensitivity by DIF. IgA CS/ICS antibodies characterize IgA pemphigus. IgA CS/ICS antibodies may be observed in nonclassical pemphigus variants along with IgG CS/ICS antibodies, intercellular IgG/IgA dermatosis.** (Lower DIF photomicrographic image demonstrates CS/ICS antibody reactivity characteristic of pemphigus disorders). **In paraneoplastic pemphigus, DIF testing on skin or mucosal biopsy specimens demonstrates IgG BMZ and IgG CS/ICS localization because of antibodies to various epithelial adhesion targets. Mixed patterns of antibody reactivity also can be found in overlapping disease presentations, including drug reactions. (B)** In the pemphigoid disorders, IIF testing **of patient serum with both split skin substrate, also termed salt-split skin (SSS), and monkey esophagus (ME) substrate demonstrates IgG BMZ antibody reactivity. Testing for IgG4 or other IgG subclass reactivity may increase detection sensitivity by IIF. Serum IgG BMZ antibodies, in bullous pemphigoid and certain other pemphigoid variants with antibodies to hemidesmosomal and lamina lucida BMZ proteins, localize to the epidermal side (roof) with SSS and also may localize in a combined epidermal-dermal pattern (roof and floor) with SSS. IgG BMZ antibodies in EBA, bullous LE, and certain other pemphigoid variants with antibodies to lamina densa basement membrane proteins, localize to the dermal side (floor) with SSS.** [Upper BMZ IIF photomicrographic images demonstrate epidermal (roof) and dermal (floor) BMZ antibody reactivity with SSS. Middle BMZ IIF photomicrographic image demonstrates combined epidermal-dermal (roof and floor) BMZ antibody reactivity with SSS. Lower BMZ IIF photomicrographic image demonstrates IgG BMZ antibody reactivity with monkey esophagus (ME) substrate, characteristic of pemphigoid (of note, the same IgG BMZ reactivity pattern is observed on ME substrate in sera from patients with EBA and bullous LE)]. **In linear IgA disease, IIF testing of patient serum with both SSS and ME substrates demonstrates IgA BMZ antibody reactivity. Serum IgA BMZ antibodies, most commonly, localize to the epidermal side (roof) with SSS, and, less commonly, to the dermal side (floor) with SSS or in a combined epidermal-dermal pattern on SSS (roof and floor). IgG and IgA BMZ antibodies may be coexpressed and are found together in sera of patients who have mucous membrane pemphigoid, IgG/IgA pemphigoid, other pemphigoid variants, and linear IgA/IgG bullous dermatosis.** In the pemphigus disorders, IIF testing **of patient serum with both ME and intact normal skin (NS) substrates demonstrates IgG epithelial CS/ICS antibody reactivity in a net-like pattern similar to the DIF findings in patient skin. IgG CS/ICS antibodies are found in sera of patients who have pemphigus foliaceus, pemphigus vulgaris, and other IgG pemphigus variants. Testing for IgG4 or other IgG subclass reactivity may increase diagnostic sensitivity by IIF. IgA CS/ICS antibodies are found in patient sera who have IgA pemphigus. Both IgG and IgA CS/ICS antibodies are found in sera from patients who have intercellular IgG/IgA dermatosis and other nonclassical types of pemphigus.** [Upper cell surface IIF photomicrographic image demonstrates CS/ICS antibody reactivity with intact NS substrate. Lower cell surface IIF photomicrographic image demonstrates CS/ICS antibody reactivity with ME substrate (distinct from IgG BMZ antibody reactivity with ME substrate found in pemphigoid)]. **In paraneoplastic pemphigus, IIF testing on SSS, NS, and ME substrates demonstrates IgG BMZ and IgG CS/ICS localization because of antibodies to various epithelial adhesion targets. Mixed patterns of antibody reactivity also can be found in overlapping disease presentations and drug reactions.**
SSS**, split skin substrate/salt-split skin;** NS**, normal skin;** ME**, monkey esophagus.**

including IgG BP180 antibodies in pemphigoid and IgG desmoglein antibodies in IgG-variant pemphigus. ELISA testing also is helpful in distinguishing disease variants, particularly for determining pemphigus subtypes; IgG desmoglein 1 autoantibodies predominate in patients with PF, and IgG desmoglein 3 autoantibodies, with or without accompanying desmoglein 1 autoantibodies, predominate in patients with PV (9). Overlapping expression with autoantibodies to both desmogleins 1 and 3 is associated clinically with features of both subtypes. However, clinically relevant antibodies may not be found by ELISA because of the restricted display of potential target epitopes in the assays. Moreover, in several dermatologic diseases, the antigenic profile has not yet been defined or ELISAs are not yet available, such as in linear IgA disease (LAD) and variants of pemphigoid. Diagnostic challenges in detecting circulating antibodies to targeted antigens also can be attributed to low levels of autoantibodies in sera and to multiple heterogeneous autoantigens, as in mucous membrane pemphigoid (MMP). Serum testing alone, either IIF or ELISA, can yield false positive results, found in various studies at 3%–7% (16–18).

Together, DIF tissue testing along with IIF and ELISA serum testing are highly sensitive and important diagnostic tools in the characterization of dermatologic disorders with autoantibodies (19–23). Supplemental Table 4 shows the predictive value of DIF and IIF diagnostic tests based on sensitivity and specificity. Testing for certain subclasses of immunoglobulins, particularly IgG4, may enhance sensitivity in DIF and IIF testing and may be missed with tests as they currently exist (24). Of note, IgG4 may contribute to the pathogenesis of immunobullous diseases (25). Figure 2 diagrammatically represents BMZ and CS/ICS antibody reactivity in autoantibody-associated dermatologic diseases determined by DIF tissue findings and IIF serum findings with representative immunofluorescence images of characteristic patterns and additional images in Supplemental Figs 1–7.

Each of the methods for diagnostic testing comes with inherent technical limitations affecting the analytical sensitivity and analytical specificity of the assay. In addition, other biological and therapeutic variables can potentially influence the testing, including intrinsic and extrinsic factors. These range from patient's immunoglobulin levels, specific autoantibody levels, and other autoantibodies to infections to treatment to substrate variability (26). Immunoassays are relatively susceptible to interference. Further explanation is provided in the online Data Supplement.

In patients who are followed over time, shifts in autoantibody levels and specificities may occur as exemplified in Fig. 3. One patient, who initially only had positive DIF tissue findings, developed fluctuating serum antibodies with differing BMZ specificities. Another patient initially had an antibody profile supporting a diagnosis of PV, transitioned over time to PF. The concept of epitope spreading is used to describe the induction, extension, or transition of an autoimmune response against host antigens and epitopes as a consequence of their exposure during immune-mediated tissue inflammation (27). Based on this phenomenon, monitoring antibody profiles in patients with autoimmune disorders is important in their disease management. Another consideration is that patients may present with more than one autoantibody-associated dermatologic disease and demonstrate mixed or atypical antibody profiles. These are becoming more common as immune-modulating agents are used successfully in treating malignancies, a notable example being the development of bullous skin diseases in patients receiving immune-checkpoint inhibitors, programmed cell death protein 1 (PD-1), and programmed cell death ligand-1 (PD-1L) blocking agents (Supplemental Table 5) (28).

AUTOIMMUNE DERMATOLOGIC DISEASES BY AUTOANTIBODY LOCALIZATION

Many of the autoimmune dermatologic diseases were named without knowledge or regard to antibody associations. Some diseases, such as pemphigoid and pemphigus, unfortunately, have nearly identical names making it seem as though they are etiologically related; often they are interchanged and referred to inappropriately (29, 30). Naming conventions of newly recognized diseases more logically have borrowed from the antigenic targets and/or autoantibody localization and/or general disease group. Although the cadre of currently recognized autoantibody-associated dermatologic diseases have an assortment of names, the diseases themselves can be broadly grouped by the location of the epithelial components targeted by the autoantibodies with some common clinical characteristics, particularly blister formation (Table 1, Fig. 1). "Tense bullae" are described in BMZ antibody-associated diseases and distinguished from "flaccid bullae" in CS/ICS antibody-associated diseases.

Basement Membrane Zone (BMZ) Antibodies

Epithelial BMZ antibody-associated diseases target hemidesmosomal and other adhesion molecules that are in or near the BMZ (Fig. 1, A). The binding of antibodies to antigens within the epithelial BMZ is associated with a disruptive inflammatory cascade that ultimately results in separation of the epidermis from the dermis in skin and epithelium from subepithelial tissue in mucous membranes. The separation typically manifests as tense blisters with or without surrounding urticarial and eczematous inflammatory patches, presumably consequent to the inflammatory cascade. Urticarial and eczematous patches also develop separately without blisters. Mucosa often is affected where blisters do not persist long due to sheer forces with mastication, leaving erosions.

Pemphigoid, Including Bullous Pemphigoid (BP) and Mucous Membrane Pemphigoid (MMP). BP is the most common immunobullous disease and, as such, the most common pemphigoid variant. It mainly affects older adults and characteristically is pruritic during all stages, prodromal preblistering, blistering, and nonblistering. Some patients experience pruritus alone. Blistering develops on all skin areas, but typically on flexural skin. MMP is the second most common pemphigoid variant, and, as its name implies, predominantly affects mucosa. MMP is a heterogeneous group of BMZ antigen-targeted disorders. Both BP and MMP can present with skin and mucosal involvement. Unlike BP, scarring sequelae often develop in patients with MMP, which has been referred to as cicatricial pemphigoid. Although still considered a rare disease, the incidence of pemphigoid may be increasing. Multiple factors are attributed to the increased incidence, including the aging population and associated increase in neurologic disorders that may have pathogenic links, increased use of drugs implicated in drug-induced disease, and an increase in the diagnosis of pemphigoid variants including localized and nonbullous pemphigoid (31).

Pemphigoid and pemphigoid-variant diseases share common findings by DIF, particularly linear IgG and/or C3 localization along the BMZ. Analysis of the linear BMZ antibody pattern by DIF provides clues for diagnosis. An n-serrated pattern has been associated with BP and MMP, whereas a u-serrated pattern has been described in disorders with antibodies to type VII collagen, including EBA and bullous LE (Supplemental Fig. 1) (32). DIF is the most sensitive diagnostic test for pemphigoid and its variants; however, the various BMZ antibody-associated diseases cannot be further characterized on biopsy alone (20).

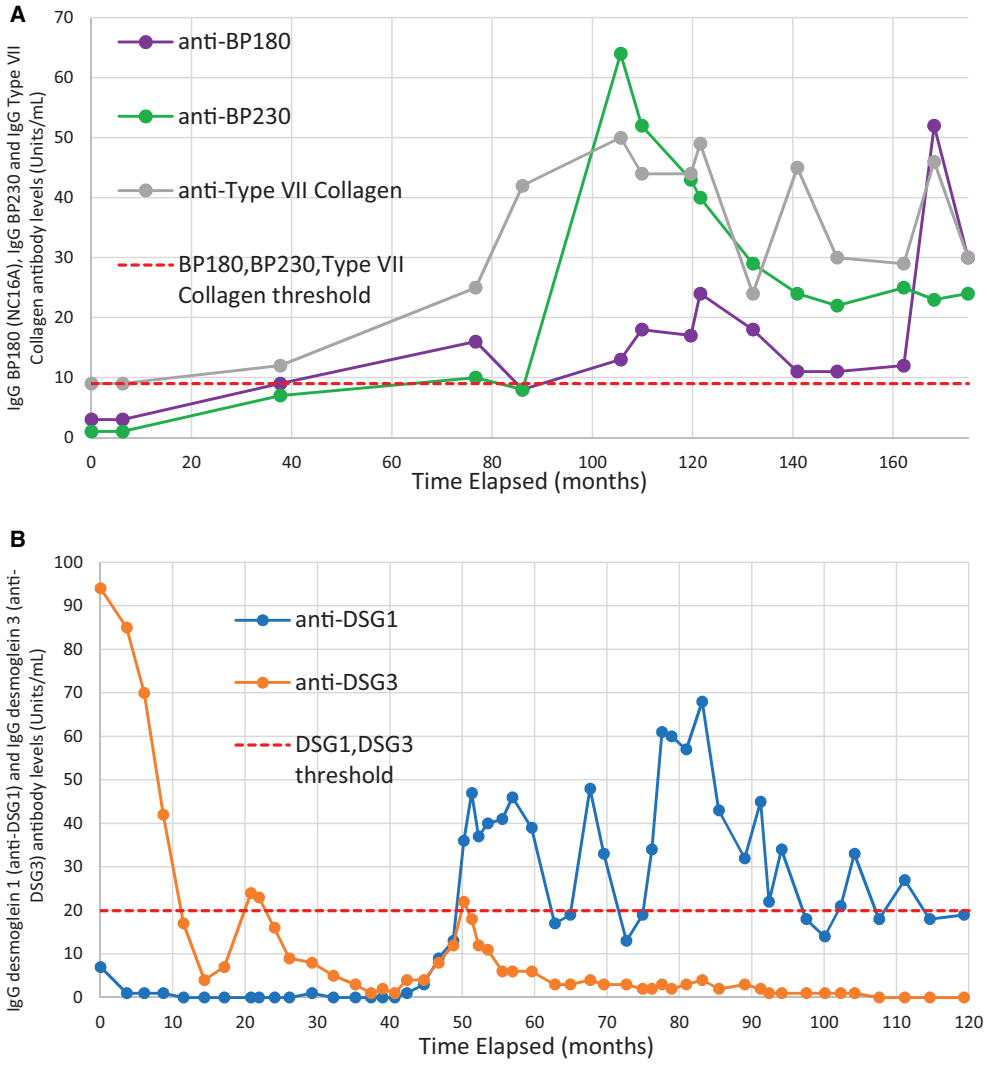

Fig. 3. Autoantibody expression profiles in dermatologic diseases over time. (A) Basement membrane zone (BMZ) antibody levels by ELISAs demonstrating developing antibody expression, evolving antibody specificities, and overlapping antibody expression over 15 years in a patient with blistering cutaneous lesions of scalp and mucosa presenting initially at time 0. Direct immunofluorescence (DIF) findings on a scalp biopsy specimen at presentation demonstrated 3+ linear IgG BMZ, 2+ linear IgM BMZ, and 2+ linear IgA BMZ antibodies, 2+ discontinuous linear C3 and 3+ linear to shaggy fibrinogen along the BMZ. Line graph displays semiquantitative ELISA units for IgG BP180 (NC16A) antibody levels (purple), IgG BP230 antibody levels (green), and IgG type VII collagen antibody levels (gray). The threshold level for increased IgG anti-BP180 (NC16A), anti-BP230, and anti-type VII collagen in the serum is indicated by the dashed red line (9 Units/mL and greater for all 3 ELISAs, MBL-Bion ELISA test kits). (B) Epitope spreading antibody reactivity to desmoglein 1 (DSG1) and desmoglein 3 (DSG3) in a patient with pemphigus demonstrating transition in antibody-directed targets from IgG DSG3 antibodies, characteristic of pemphigus vulgaris, to IgG DSG1 antibodies, characteristic of pemphigus foliaceus. Line graph displays semiquantitative ELISA units for serum IgG DSG1 (blue) and IgG DSG3 (orange) antibody levels in a patient over 10 years. The threshold level for increased IgG anti-DSG1 and IgG anti-DSG3 in serum is indicated by the dashed red line (20 Units/mL and greater for both ELISAs, MBL-Bion ELISA test kits).

Serum evaluation, by both IIF and ELISA, plays an integral role in further defining BMZ antibody-associated diseases. The differentiation of pemphigoid subtypes has significant clinical implications because treatment considerations, prognosis, and association with other underlying conditions or medications vary among the disorders. IIF testing on split skin substrate (also known as salt-split skin) typically is performed in the initial differentiation. Epidermal predominant (roof) or combined epidermal-dermal (roof and floor) localization of IgG BMZ antibodies is observed with serum testing on split skin substrate from patients with BP. Up to half of patients with MMP are negative by serum IIF testing, without detectable circulating BMZ antibodies. Of those patients who are positive for IgG BMZ antibodies on split skin substrate, most show epidermal (roof) localization (33). The epidermal localization (roof) pattern reflects the presence of antibodies to antigens within or above the lamina lucida of the BMZ, including BP180, BP230, alpha6beta4 integrin (Fig. 1). Dermal (floor) localization of IgG antibodies on split skin substrate by IIF defines 4 BMZ antibody-associated disease subtypes: EBA and bullous LE with antibodies to type VII collagen; anti-p200 (laminin gamma-1) pemphigoid, and anti-laminin-332 pemphigoid. Categorically, dermal BMZ antibody localization (floor of salt-split skin) has been equated with the diagnosis of EBA; however, a recent study of serum specimens with dermal IgG antibody reactivity on split skin substrate revealed that most (82%) had antibodies directed to p200/laminin gamma-1, whereas considerably smaller subsets demonstrated type VII collagen (11%), laminin-332 (9%), and undefined (6%) antibodies (34).

Patients with anti-p200 pemphigoid are typically younger than those with BP and have lesions that clinically resemble both BP and the inflammatory EBA variant (see next) (35). In one early series, approximately half the patients had coexisting psoriasis and were mainly male (36). Although the target antigen originally was identified by immunoblotting as a 200 kDa protein extracted from human dermis, subsequently, approximately 90% of patients with this pemphigoid subtype were found to have antibodies to laminin gamma-1 (37). Mucous membrane involvement is predominant in anti-laminin-332 pemphigoid. Recognition of the association of this pemphigoid variant with underlying or developing malignancy (typically solid tumor) in up to one-third of cases is critical, and highlights the importance of differentiating the dermal-predominant pemphigoid subtypes so appropriate clinical evaluation is conducted (38, 39). Although clinical laboratory testing for antibodies to laminin-332 is not readily available currently, an IIF assay using human embryonic kidney-293 (HEK293) cells expressing recombinant laminin-332 has been developed for detection of these antibodies (Euroimmun FA 150 b-50) (14).

Various drugs exposures, 50 or more, have been reported to induce pemphigoid, although, in most cases, the strength of an association is indeterminate (Supplemental Table 5) (40, 41). BP also has been reported with infections, radiation, and other physical exposures, in the setting of renal disease with BMZ antibodies, and following inflammatory diseases such as Stevens Johnson syndrome and lichen planus (LP), possibly through exposure of BMZ antigens with the illnesses. BP has been reported in association with neurologic disorders, and the homology between BP antigens in the skin and neuronal antigens in the central nervous system, along with a genetic predisposition, is postulated to explain the observed link (42). Certain human leukocyte antigen (HLA) alleles (43) may contribute to disease risk by the facilitation of BMZ and/or neuronal antigen presentation to T cells (44).

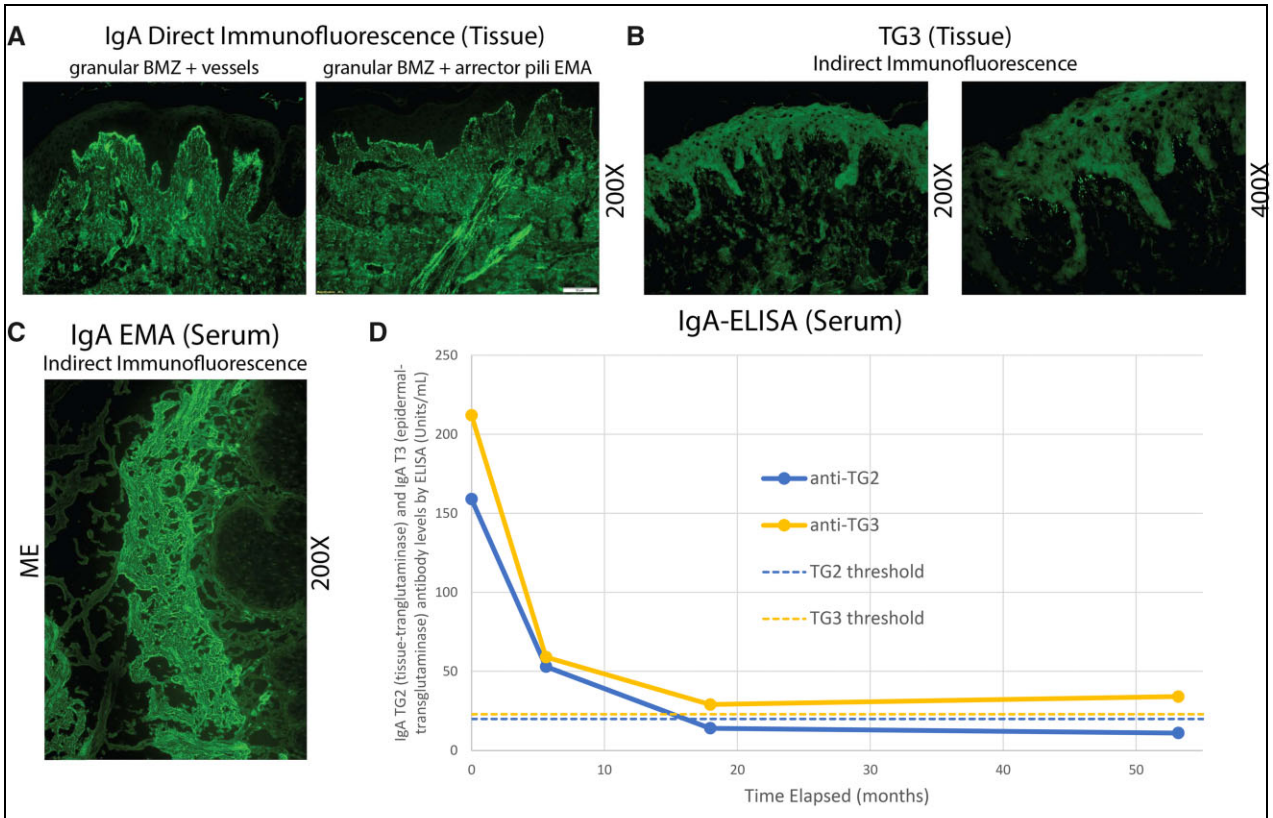

Fig. 4. Autoantibody expression in dermatitis herpetiformis (DH). (A) **Photomicrographic images of skin biopsy specimens tested by direct immunofluorescence (DIF) demonstrating characteristic and strong IgA in upper dermis, granular pattern along the basement membrane zone (BMZ) with stippling in dermal papillae in both. The left image also demonstrates vascular IgA, as can be observed in DH, and the right image also demonstrates IgA endomysial antibody (EMA) reactivity in arrector pili, reflecting serum IgA EMA as found associated with celiac disease (CD).** (B) **Photomicrographic images of a skin biopsy specimen tested by indirect immunofluorescence (IIF) for epidermal transglutaminase (eTG), also known as transglutaminase 3 (TG3), demonstrating distinctive expression of TG3 in upper dermis, to which IgA antibodies are directed in DH. The right image is a higher power view of the left TG3 image and also reveals the relatively sparse granularity along the BMZ that often is found in DH by DIF.** (C) **Photomicrographic image of serum tested by IIF demonstrating IgA EMA on smooth muscle endomysium present in distal monkey esophagus as substrate (the distal third of the esophagus in primates has a smooth muscle band around the epithelium). Most patients with untreated DH have positive IgA EMA in their sera by IIF as are characteristically found in patients with CD. EMA also can be detected on human umbilical cord substrate (Supplemental Fig. 2 is a photomicrographic image with positive IgA EMA on umbilical cord substrate), and, as above in the right DIF panel, on arrector pili smooth muscle.** (D) **Graph of serum levels of IgA tissue transglutaminase (tTG), also known as transglutaminase 2 (TG2), and IgA TG3, by ELISAs in a patient with DH over time reflecting decreased levels after diagnosis and while following a gluten-free diet. TG2, is the main antigenic component of endomysium to which antibodies develop, and increased IgA TG2 antibody levels, determined by ELISA, as well as positive IgA EMA titers, determined by IIF, correlate with disease activity in individual patients with DH and CD. TG3 is the dominant antigen to which IgA antibodies develop in DH, and levels of IgA TG3 antibodies, determined by ELISA, may correlate with disease activity in individual patients. Patients with DH can demonstrate an antibody profile specific for TG3 with higher avidity than to TG2. The threshold levels for increased IgA TG2 and IgA TG3 in the serum are indicated by the dashed lines for each ELISA (20 Units/mL and greater for IgA TG2, Inova ELISA kit; and 23 Units/mL and greater, with 16–22 Units/mL in a borderline/indeterminate range, for IgA TG3, Immundiagnostik AG ELISA kit).**

Pemphigoid Gestationis (PG). Pemphigoid gestationis, a rare pregnancy-associated dermatosis characterized by pruritic papules, urticarial plaques, and blisters, shares immunopathologic features with BP (45). PG is associated with risk for preterm delivery and small-for-gestational age babies and recurs with subsequent pregnancies. Prompt, accurate diagnosis is important for therapeutic management and to distinguish it from other pregnancy-associated dermatoses that may show clinical overlap and may require other therapeutic interventions for a viable term pregnancy (46). The diagnosis of PG is supported by DIF findings on a perilesional skin biopsy specimen showing strong linear C3 BMZ localization with weaker or absent linear IgG BMZ reactivity. Similarly, serum testing typically demonstrates either low titer or negative IgG BMZ antibody reactivity by IIF; however, ELISA testing reveals increased IgG BP180 antibody levels with antibodies directed against the NC16A domain, the antigenic domain shared with BP (47). Additional serum testing with a specific IIF modification to add a fresh source of complement and with ELISA for IgG BP180 antibodies reveals complement-fixing BMZ antibodies (historically termed, herpes gestationis factor or HGF) against BP180. IgG BP180 antibodies may correlate with disease activity in patients with BP but remain increased in patients with PG even with disease remission/resolution. A subset of patients with PG also develop serum autoantibodies characteristic of celiac disease (48).

Epidermolysis Bullosa Acquisita (EBA). EBA is regarded as the prototypic subepidermal immunobullous disease with dermal pattern antibody reactivity on split skin substrate. EBA is a chronic blistering disorder demonstrating a heterogeneous clinical picture with 6 variants. Despite the variable clinical presentations, all EBA variants express antibodies directed at type VII collagen. Type VII collagen is a major component of anchoring fibrils attaching the epidermis to the underlying dermal connective tissue (Fig. 1) and is composed of 3 identical alpha chains, each with a collagenous domain flanked by 2 NC domains, NC1 and NC2. Antibodies most commonly are directed at the NC1 domain, while the NC2 domain is a minor antigenic target. ELISA testing for antibodies directed against the NC1 and NC2 domains use commercially available kits and, along with IIF on split skin substrate, are performed for the diagnosis of EBA (49). Patients with inflammatory bowel disease, including Crohn's disease and ulcerative colitis, with and without mucocutaneous manifestations of EBA, may demonstrate increased levels of antibodies to type VII collagen.

Bullous Lupus Erythematosus (LE). Up to two-thirds of patients with bullous LE also demonstrate antibodies to type VII collagen positioning the diagnosis as a BMZ antibody-associated disease. Patients with bullous LE typically present with systemic disease, including lupus nephritis, and often demonstrate multiple positive lupus-associated serologic markers including antinuclear antibodies and anti-dsDNA antibodies (50). DIF testing provides clues that are diagnostically helpful in distinguishing bullous LE from other BMZ antibody-associated diseases by the presence of multiple immunoglobulin classes (IgG, IgM, and IgA) showing granular immune deposits along the BMZ. Three types of bullous LE have been described: type I is the most common with autoantibodies targeting the NC1 and NC2 domains of type VII collagen; in type II, autoantibodies target other antigens of the BMZ, including BP180, BP230, and laminin-332 or laminin-331; and type III demonstrates uncharacterized epidermal, with or without dermal, reactivity (50).

Linear IgA Disease (LAD). LAD is a subepidermal, vesiculobullous eruption with lesions resembling pemphigoid and DH that occurs in adults and

children (51). Until recently, childhood LAD was called "chronic bullous disease of childhood," and adult LAD was called "linear IgA bullous dermatosis." Both childhood and adult presentations are characterized by the linear deposition of IgA along the BMZ by DIF, the defining basis of the current diagnostic name for both, linear IgA disease/LAD (52). LAD has been reported in association with medications, infections, malignancies, connective tissue diseases, and inflammatory bowel disease, particularly ulcerative colitis. Vancomycin is the most commonly implicated drug in LAD, other medications have been reported (Supplemental Table 5).

Two distinct types of LAD are recognized by the localization pattern of serum IgA antibodies on split skin substrate by IIF: a lamina lucida type with epidermal (roof) localization and a sublamina densa type with dermal (floor) localization (53, 54). The lamina lucida type is considerably more common (91%) than the sublamina densa type (9%) (55). The sublamina densa type is more refractory to treatment. Linear IgA BMZ in a tissue biopsy by DIF shows an n-serrated pattern in the lamina lucida type and a u-serrated pattern in the sublamina densa type (Supplemental Fig. 1). IgA antibodies of the lamina lucida LAD type react with 2 proteolytically processed truncated ectodomains of BP180 (Fig. 1, B) that express neoepitopes (56). Sera from patients with LAD preferentially react on immunoblot with 2 fragments, LAD1, a 120-kD fragment (57), and LABD97, a 97-kD fragment, but not intact BP180 (58). Less commonly, sera from patients with LAD react with the NC16 domain of BP180. Most LAD antibodies of the sublamina densa type react with type VII collagen, as characteristically observed in EBA with IgG antibodies (55), while only rare LAD antibodies demonstrate laminin gamma-1 reactivity (54).

Some patients may have both linear IgA and IgG BMZ antibodies. This is referred to as either LAD or pemphigoid, based on the predominant immunoglobulin class detected, or as linear IgA/IgG bullous dermatosis (59). Patients whose serum has both IgA and IgG BMZ antibodies may have more severe disease and/or show different responses to therapies.

Other Findings Pertinent to BMZ Autoantibody Reactivity. Assessment of BMZ by DIF can identify findings associated with other nonautoantibody blistering disorders. In particular, porphyria cutanea tarda and pseudoporphyria show DIF findings similar to EBA, bullous LE, and LAD, and even a resemblance to vasculitis. Porphyria and pseudoporphyria characteristically demonstrate homogenous immunoglobulin, complement, and fibrinogen thickened BMZ and perivascular staining by DIF in sun-exposed skin (Supplemental Fig. 7); moreover, a u-serrated, also referred to as pseudo-u-serrated, BMZ pattern along with vascular staining have been described in these disorders. The findings could obfuscate weak or subtle specific disease-defining antibody reactivity, given their locations. Careful DIF examination reveals subtle differences in the BMZ staining patterns, but overlapping features are present, and the integration of clinical and all laboratory data is important in the diagnostic assessment (60).

Cell Surface (Intercellular Substance or ICS) Antibodies

Epithelial cell surface (CS) antibody-related diseases target adhesion proteins in keratinocyte desmosomes (Fig. 1, C) reducing adhesion between epithelial cells by interfering with the binding functions of the proteins. The disease mechanisms that produce loss of keratinocyte adhesion, termed acantholysis, and disease expression are complex with strong genetic factors (61, 62). Exogenous exposures likely play a role, most apparent in endemic pemphigus (fogo selvagem) and drug-induced pemphigus.

Pemphigus. Pemphigus is the rare, severe autoantibody-related group of skin and mucous

membrane diseases in which expression of antibodies to desmoglein and desmocollin cadherins leads to intraepithelial separation (Fig. 1, C and Supplemental Fig. 8). The flaccid bullae that ensue rarely remain intact, and large, painful erosions are prominent in the mucosa and/or skin with fine corn flake-like skin scaling. Untreated, the process persists and progresses. Depending on the extent of mucosal and skin involvement, the loss of epithelial integrity can be life-threatening. Pemphigus variants are phenotypically defined by the location of the targeted cadherin antigens in the epidermal/mucosal desmosomes and the spectrum of dysfunction induced by the pemphigus autoantibodies (Table 1).

DIF findings on biopsy tissue and serum antibody determinations by IIF and ELISA testing (Tables 1 and 2) are important for diagnosis and for monitoring disease activity. DIF testing demonstrates intercellular IgG localization in the epidermis/epithelium in a net-like or punctate pattern (Supplemental Fig. 1), typically also C3 or C3 alone in the same pattern, in IgG pemphigus variants, and IgA intercellular localization in the rare IgA pemphigus variants (63, 64). Serum testing by IIF broadly characterizes the autoantibody pattern of CS/ICS staining that distinguishes pemphigus from pemphigoid and other disorders with BMZ autoantibodies and establishes the immunoglobulin class, IgG and/or IgA, of the antibodies (Fig. 2). Limiting-dilution, end-point antibody titers by IIF correlate with disease activity. ELISA testing is widely available for IgG desmoglein 1 and IgG desmoglein 3 antibodies, the main targeted cadherins in pemphigus, and is highly sensitive, with >90% of patients with pemphigus showing increased levels of one or both antibodies. IgG desmoglein antibody levels also correlate with disease activity. Of note, patients with CS/ICS antibody-positive pemphigus by DIF and IIF can have normal results on ELISA testing because of antibodies to different desmoglein 1 and/or desmoglein 3 epitopes than displayed in the ELISAs or to other adhesion molecules. IgG antibody testing to other targeted adhesion molecules in pemphigus or IgA pemphigus antibody testing are not available except in research settings, although assay development for these is forecast.

PV, with predominant IgG antibody expression to desmoglein 3, mainly involves the mucosa where desmoglein 3 is abundant in the epithelium; PF, with IgG antibody expression to desmoglein 1, mainly involves the skin where desmoglein 1 is present, most intensely in the upper epidermis with lesser desmoglein 3. Antibodies to both desmoglein 1 and desmoglein 3 are linked to mucocutaneous disease. IgG antibodies to desmocollin 3 may contribute to pemphigus in some patients along with antibodies to many other adhesion targets, including desmoglein 2, the acetylcholine receptor, pemphaxin, and others (65). Drug-induced pemphigus immunopathologically resembles PF and, less commonly, pemphigus erythematosus, PV, or paraneoplastic pemphigus (PNP). IgA pemphigus demonstrates IgA antibody expression to desmocollin 1 in subcorneal pustular dermatosis and IgA antibody expression to desmogleins 1 and 3 in intraepidermal neutrophilic dermatosis. Other variants are described with autoantibody profiles that align with one of the main variants listed previously or with combined antibody features, as exemplified by pemphigus erythematosus (Senear–Usher syndrome) with features of PF and LE, now considered PF. IgA, along with IgG, cell surface antibodies in tissue by DIF and in serum by IIF may be observed in nonclassical pemphigus and in intercellular IgG/IgA dermatosis (66).

A confounding factor in IIF testing for pemphigus is the presence of pemphigus-like antibody reactivity that can make interpretation of findings difficult. This cell surface staining tends to be weaker and less sharply defined than that typically observed with pemphigus antibodies, in low titer, and may be transient. Pemphigus-like immunostaining has been found in various skin afflictions

including burns, infections, drug reactions, LE, toxic epidermal necrolysis, contact dermatitis, other mucocutaneous disorders, and myasthenia gravis. It has been postulated to result from nonspecific cross-reactivity to blood group products. It also has been thought to be a temporary and secondary phenomenon from provoking factors that alter and expose epithelial antigens, as in burn trauma, or evidence of a predisposition to pemphigus, heralding the potential for pemphigus development with long-term exposure to an inducing factor, such as certain drugs (Supplemental Table 5).

Cell Surface and BMZ Antibodies

Immunopathologic induction of CS/ICS and BMZ antibody expression is associated with clinical features recognized in each separate phenotype and uniquely together. Considerations for mixed and atypical epithelial autoantibody expression profiles include: concurrent disease presentations with codominant autoantibody expression; incidental crossover epithelial antibodies with dominant features of one immunobullous disease; autoimmune diseases in patients who are multiple autoantibody producers; nonspecific expression of one or more of the antibodies; drug reactions; epithelial antibodies to cross-reacting antigens in patients with neurologic diseases or other disorders, including neoplastic/malignancy-associated conditions in addition to or other than PNP.

Paraneoplastic Pemphigus (PNP). Both cell surface and BMZ autoantibodies characteristically develop in patients with PNP, identified in biopsy specimens by DIF and in serum by IIF on rodent bladder substrates (Supplemental Fig. 3). Depending on the lesional morphology, lichenoid features (see later for description) also may be observed by DIF with cytoid body formation and/or shaggy fibrinogen BMZ deposition (Supplemental Fig. 5). Autoantibodies in patients with PNP target multiple antigens in all epithelial types, but predominantly to plakins (proteins that link cytoskeletal elements to each other and to specialized plasma membrane sites to provide mechanical strength and ensure proper cellular development) (Fig. 1). Bladders are particularly good substrates for PNP antibody testing, presumably because the highly conserved desmoplakin adhesion molecules are well-represented. Rat bladder has become a standard substrate for PNP antibody IIF testing with relatively high specificity but relatively low sensitivity, based on comparable murine bladder testing (67). The sensitivity can be improved with a complement fixation modification, as performed for PG (68). Negative PNP antibody testing by IIF does not rule out paraneoplastic/malignancy-associated disease. Patients with IgA PNP antibodies, both with and without IgG, by IIF have rarely been reported with lesions similar to IgG PNP (69, 70).

PNP presents with various lesion types (Table 1). PNP affects all ages and develops in association with malignancies, most often hematologic (lymphoma, leukemia) and sarcoma. It also may accompany benign neoplasias, especially Castleman's, which is the most frequent association in children and adolescents. Based on the myriad epithelial antigens that PNP antibodies target (Table 1), in addition to mucocutaneous involvement, various organs can be affected, including lungs, gastrointestinal tract, kidney, and thyroid. The development of bronchiolitis obliterans or myasthenia gravis portends increased mortality. To reflect the range of potential organ involvement, this autoimmune reaction is regarded by a more inclusive name in some publications, paraneoplastic autoimmune multiorgan syndrome (71). Because an underlying neoplasm is almost always present, any patient suspected of having PNP, but no previously detected neoplasm, should undergo a prompt, thorough evaluation for malignancy (72, 73).

Consideration of antibody testing for targeted analytes by ELISA or other methods is daunting in PNP with the number of possible epithelial

antigens (Table 1). ELISA testing for IgG envoplakin antibodies, with high specificity (96%–99%) and sensitivity (80%–86%), is available through some laboratories. IgG envoplakin antibody levels correlate with extent of mucocutaneous PNP disease. After successful tumor therapy, envoplakin antibody levels decrease significantly. Patients with PNP also may develop antibodies to desmogleins 1 and 3 and to BP180 and BP230, and the results from these ELISAs can provide additional immunopathological information about the autoantibody profile (72).

Other Mixed Autoantibody Presentations. Many concurrent disease presentations with mixed autoantibody expression have been described and are detected in diagnostic testing. Patients are reported who demonstrate clinical and immunopathological features of more than one autoanti body-associated disease, such as pemphigus together with pemphigoid or pemphigoid together with DH. There also are patients with overlapping antibody expression, likely incidental and/or nonspecific, and others coexpress antibodies of two immunoglobulin classes. Certain drugs may be inducing factors for more than one autoantibody-expressing diseases, especially with immune checkpoint inhibitors. (Supplemental Table 5). By activating various immunological mechanisms, infections can be inciting factors. As noted, tumor antigens and neurologic diseases share common antigenic targets with autoantibodies in skin diseases (42).

Patients with various autoimmune disorders have an immunologic propensity to develop autoantibodies to multiple antigenic targets, including disease-defining autoantibodies and others of uncertain clinical relevance. The scope of this review does not include potential genetic relationships among the autoantibody-associated dermatologic diseases, but certain HLA class II genes are prevalent and common in patients with these disorders. HLA class I and II gene clusters are involved in antigen processing and presentation, show high significant associations with autoimmune diseases, and represent strong predisposing genetic factors for autoantibody development.

MISCELLANEOUS AUTOANTIBODY-ASSOCIATED DISEASES

DH is an autoantibody-associated disorder with both skin and intestinal manifestations and characteristic DIF, IIF, and ELISA findings. Various other inflammatory skin disorders, such as LP, cutaneous LE, and vasculitis, demonstrate characteristic DIF patterns that are consistent and recognizable and provide diagnostic value.

Dermatitis Herpetiformis (DH)

DH is an intensely pruritic skin disease induced by gluten ingestion and demonstrating papules, vesicles, and excoriations on extensor skin surfaces. By DIF, DH is characterized by subepidermal granular IgA deposition in skin biopsy specimens and variable severity of gluten-sensitive enteropathy, otherwise identical to celiac disease (CD) (74). DH may present before CD is recognized and, therefore, is important to diagnose promptly to minimize nutritional deficiencies and risks for intestinal lymphoma and to distinguish from other epithelial autoantibody-associated disorders.

DIF findings in normal-appearing skin immediately adjacent to a lesion demonstrate granular (90%) or fibrillar (10%) IgA in dermal papillae and upper dermis (75). Because IgA may be deposited in a patchy distribution in and around lesions, DIF findings on biopsy specimens may demonstrate sparse or no IgA, and serial sectioning or multiple biopsies may be needed to convincingly observe it. Granular IgA also may be found in the walls of superficial dermal blood vessels by DIF (76). Grains noted in the smooth muscle of the arrector pili by DIF are a reflection of IgA endomysial antibodies (EMA) in the circulation. Characteristic immunopathological findings in DH are shown in Fig. 4.

DIF is needed to differentiate DH from LAD and bullous LE, which show similar histopathology with neutrophilic infiltration in dermal papillary tips (approximately 60% of patients) and vesicle formation at the dermal–epidermal junction. Differentiating these disorders is essential for management because the other disorders do not characteristically respond to dietary gluten restriction (77). In 65% of patients with CD without skin disease, granular IgA deposits are observed in normal skin by DIF, but are not found in patients with other gastrointestinal disorders who have skin conditions or in normal individuals, indicating the possibility of a latent marker for DH (78).

Passive transfer experiments showed that IgA antibodies targeting epidermal transglutaminase (eTG), also known as transglutaminase 3 (TG3), are responsible for the characteristic granular IgA reactivity by DIF (79). Testing biopsy specimens for TG3 by an IIF procedure is positive when prominent IgA localization is found. Fibrinogen, in a granular or aggregated pattern, is commonly observed in the same distribution as IgA and is likely involved pathogenically in lesion development (80). Granular IgG (16%), IgM (61%), and C3 (61%) deposits along the BMZ also may be found with the characteristic granular IgA (81). When granular immune deposits stain for multiple conjugates by DIF, the findings overlap with those observed in LE.

Most patients with DH have CD to some degree with characteristic serologic findings, although the intestinal inflammation often is less severe in patients with DH. Serum IIF is performed to test for IgA EMA, and ELISAs to test for IgA antibodies to tissue transglutaminase (tTG), also known as transglutaminase 2 (TG2), the main autoantigen in the endomysium, and eTG/TG3, the specific autoantigen in DH (74). Positive/increased results from these tests can be used as diagnostic markers and titers/levels of the antibodies for monitoring response to dietary gluten restriction. IgA tTG/TG2 or IgA eTG/TG3 antibody negativity does not exclude a diagnosis of DH. Patients with CD with or without associated DH may have positive IgG EMA by IIF and/or increased IgG tTG/TG2 antibodies in sera, both in the presence and absence of IgA antibodies; such IgG antibodies may be present in patients with CD/DH with IgA deficiency. Antibody testing to gliadin and deamidated gliadin does not add to the testing for EMA, tTG/TG2, and eTG/TG3 antibodies in the diagnosis of DH or monitoring disease activity.

Specific HLA types are required for processing the gliadin antigen, and either HLA-DQ2 (in approximately 95%) or HLA-DQ8 (approximately 5%) is present in virtually all patients with CD and DH. HLA typing has a relatively low positive predictive value because of the high prevalence of HLA-DQ2 and HLA-DQ8 in the normal Caucasian population. This limits its utility as a disease marker, and HLA typing is not recommended as a standard diagnostic test for CD or DH. However, its high negative predictive value can be useful in certain clinical situations to eliminate CD and DH from the differential diagnosis (74).

IIF testing for IgA EMA is performed using monkey esophagus (ME) substrate, serial dilutions of patient serum to limiting-dilution, end-point titers, and fluorescein-conjugated antihuman IgA. Honeycomb-like reactivity is observed around smooth muscle fibers (Fig. 4). Between 60% and 90% of patients with DH on a gluten-containing diet will have positive IgA EMA, indicating reactivity as found in CD (74). ELISAs for IgA tTG/TG2 antibodies are commonly performed for diagnosing CD and monitoring disease activity. ELISAs may be more sensitive than IIF testing, and IgA tTG/TG2 antibody levels generally parallel IgA EMA titers. Of note, tTG/TG2 antibodies by ELISA may be increased in patients with inflammatory bowel disease and other intestinal disorders and not specifically in patients with CD with or without DH.

The presence of IgA eTG/TG3 antibodies is a strong diagnostic marker for DH. Variable sensitivity with commercially available ELISA kits and relatively low, variable specificity has limited their

clinical utility; half or more patients with CD, without dietary gluten restriction and without skin disease, have increased antibody levels. It seems possible that "false positive" findings could be harbingers of DH that will later develop in patients with preexisting CD. A new IgA eTG/TG3 assay using activated recombinant TG3 and a gut-derived human monoclonal IgA TG3 standard for calibration shows increased specificity and sensitivity as well as correlation of antibody levels with dietary gluten restriction (82).

Lichen Planus (LP) and Lichenoid Reactions

DIF findings in LP include characteristic shaggy deposition of fibrinogen along the basement membrane, reflecting inflammation at the epidermal-dermal junction, termed "interface dermatitis," and large, grouped, globular clusters, termed "cytoid bodies" (Supplemental Fig. 5). Cytoids represent dead and dying keratinocytes and degenerated basement membrane segments and show nonspecific fluorescence. The DIF combination of numerous cytoid bodies and shaggy fibrinogen BMZ deposition increases the specificity of a diagnosis of LP over the other many conditions that exhibit features of a lichenoid reaction (83). DIF testing can be particularly helpful in differentiating LP from other immunobullous disorders when only mucosal tissue is involved (84). Lichenoid reactions are found in LP, premalignant and epithelial malignancies, drug reactions, erythema multiforme, Steven's Johnson/toxic epidermal necrolysis, LE, dermatomyositis, other connective tissue disease, graft vs host disease in skin and mucosa, and other mucocutaneous disorders, including LP pemphigoides and PNP.

Cutaneous Lupus Erythematosus (LE)

Clinical and immunopathologic aspects of cutaneous LE are broad and beyond the scope of this review; however, the distinctive DIF features that may be observed in any of the cutaneous LE types and those characteristic of subacute cutaneous LE (SCLE) will be highlighted. Bullous LE mainly develops in patients who have been diagnosed with SLE, although bullous LE is the initial presentation in about a third of patients. As reviewed previously, in common with EBA, autoantibodies to type VII collagen are expressed and, therefore, bullous LE is regarded in the pemphigoid/BMZ antibody-associated diseases.

Coarse, granular, continuous deposits of immunoglobulins and complement along the basement membrane are the DIF findings in cutaneous LE. In addition, staining of epidermal nuclei may be observed reflecting antinuclear antibodies in the disease (85). A pattern of particulate epidermal IgG deposition is observed by DIF in biopsy specimens of patients with SCLE (images in Supplemental Fig. 4). Patients with SCLE characteristically have serum autoantibodies to the extractable nuclear antigen, Ro/SSA, and the distinctive pattern is thought to reflect the Ro/SSA autoantibodies (86). Cutaneous LE, including SCLE, lesions also may demonstrate lichenoid features by DIF with cytoid bodies and shaggy fibrinogen deposition along the BMZ. DIF features of antibody-mediated vasculitis may be observed in biopsy specimens from patients with lupus (see next).

DIF evaluation of lesional and nonlesional, non-sun-exposed skin for the differential presence of characteristic continuous granular immune deposits along the BMZ is historically defined as the "lupus band test." The lupus band test has been referred to variably and inconsistently in evaluating patients with skin lesions for systemic LE; in systemic LE, skin specimens from both sites demonstrate the defining DIF findings. Using the terms, "lesional lupus band test" and "nonlesional lupus band test," are helpful to indicate the tissue state identified by the test. Other connective tissue disorders and even sun-exposed skin may show similar DIF findings, but usually to a considerably lesser degree in the intensity and continuity of the granular immune deposits found in cutaneous LE. It is imperative to correlate DIF results

with clinical presentation, histopathological findings, and serologies in the diagnosis of LE. [Specific antinuclear antibody (ANA) patterns and nuclear antigens to which autoantibodies develop can be found in other reviews with availability of electronic ANA information at https://www.anapatterns.org/index.php.] (87)

Cutaneous LE presents with a range of lesions from the localized erythematous, edematous plaques of the "tell-tale butterfly"/malar rash to a toxic epidermal necrolysis-like eruption to deep, boggy tumid lesions. SCLE presents with annular and papulosquamous lesions, and, less commonly, erythrodermic, poikilodermatous, erythema multiforme-like (Rowell syndrome), vesiculobullous annular variant SCLE, and drug-induced SCLE. Any cutaneous LE can be found in patients with systemic LE (SLE). Neonatal LE shares features with SCLE and is considered to be a manifestation of SCLE, likely because of transplacental antibody transfer. Drug-induced LE commonly presents as SCLE and also as systemic LE with or without other cutaneous LE lesions (implicated drugs are listed in Supplemental Table 5) (88). Pemphigus erythematosus, also known as Senear–Usher syndrome, demonstrates clinical and immunopathological features of both PF and LE.

Several common histopathological features unite the LE-specific skin diseases and include hyperkeratosis; epidermal atrophy; vacuolar interface dermatitis (also described as liquefactive degeneration of the epidermal basal layer); superficial, perivascular, and perifollicular mononuclear cell infiltration; thickening of the basement membrane; and pigment incontinence. Some or all of the common features may be present, and the features are not exclusive to LE-specific skin disease. Interface dermatitis, in particular, also is found in skin lesions in patients with dermatomyositis and overlap and mixed connective tissue disease and is a characteristic feature of LP and lichenoid tissue reactions generally, including those with other distinctive findings, such as LP pemphigoides.

DIF findings in cutaneous LE differ from those of LP and other conditions that have similar clinical and histopathological presentations, lending value to DIF testing to differentiate these conditions. This is particularly so for SCLE. However, there are differing opinions as to the value of DIF testing in cutaneous LE skin biopsy specimens.

Vasculitis

DIF testing is an important diagnostic tool in the evaluation of vasculitis with skin involvement, although not all types of vasculitis demonstrate positive DIF findings. Skin involvement most often is found in small vessel vasculitis, manifesting as palpable purpura, erosive hemorrhagic crusting, urticarial papules, or a combination of the lesions. Medium and large vessel vasculitides are less likely to have skin manifestations. The pattern and class of immunoglobulin deposition in and around blood vessel walls and other DIF findings in skin biopsy specimens can aid in the classification of vasculitis. C3 localization in blood vessels often accompanies immunoglobulin reactivity, and fibrinogen deposition around blood vessels characteristically is strong and broad (89, 90). The predominance of IgA in superficial dermal blood vessels is the defining feature of IgA vasculitis, which can present alone in the skin or as part of the Henoch–Schönlein purpura syndrome with associated gastritis, nephropathy, and/or arthritis (91). Other patterns of vascular immunoglobulin deposition are not diagnostic for a specific type of vasculitis and additional evaluations, including hepatitis serologies, lupus serologies, complement, cryoglobulins, neutrophil antibodies, and drug history, are needed for further classification (92). It is important to obtain fresh lesional tissue for DIF when vasculitis is suspected. DIF testing of skin biopsy specimens taken from newly developed (<48 hours old) lesions of vasculitis afford the greatest likelihood of observing characteristic

findings that, theoretically, can be rapidly degraded and obfuscated by the inflammation, revealed on histopathologic examination by dense inflammatory cell infiltration around blood vessel walls, and attendant vascular destruction. Granular immune deposits along the BMZ together with vascular immunoglobulin and complement staining indicate lupus-related vasculitis or hypocomplementemic urticarial vasculitis. Immune deposits are detected in skin biopsy specimens from patients with granulomatosis with polyangiitis, with IgG and/or IgA being the most common, in and around subepidermal blood vessels (93). The potentially serious systemic manifestations of this condition make accurate diagnosis crucial.

LESS CHARACTERISTIC, LESS WELL-KNOWN CLINICAL, TYPICAL NONSPECIFIC, AND ATYPICAL AUTOANTIBODY PROFILES

The focus of this review is on diagnostics for dermatologic diseases and their characteristic autoantibody associations. Information on less characteristic presentations and antibody associations can be found in the online Data Supplement.

FUTURE DIRECTIONS

Immunodermatology testing is in its early stages with remarkable growth potential for understanding autoantibody-associated diseases and individualized, precise approaches to care. Undoubtedly, in time, artificial intelligence will have commercial application to immunodermatology testing, either on existing diagnostic platforms or newly developed technology. Certain developing diagnostic approaches are elaborated on in the online Data Supplement. Work is needed to elucidate the pathways that lead to autoantibody production and disease expression. Genetic factors, exogenous exposures, especially those producing molecular mimicry with autoantigens, and the phenomenon of epitope spreading all are potential factors. Understanding the interplay of these factors likely will lead to determining how to halt or mitigate disease induction and progression. Advancements in diagnostics for dermatologic diseases with autoantibodies assuredly will evolve.

Nonstandard Abbreviations: DH, dermatitis herpetiformis; **CD,** celiac disease; **DIF,** direct immunofluorescence; **IIF,** indirect immunofluorescence; **EBA,** epidermolysis bullosa acquisita; **BMZ,** basement membrane zone; **ELISA,** enzyme-linked immunosorbent assay; **BP,** bullous pemphigoid; **LE,** lupus erythematosus; **CS,** cell surface; **ICS,** intercellular substance; **PF,** pemphigus foliaceus; **PV,** pemphigus vulgaris; **NC,** noncollagenous; **LAD,** linear IgA disease; **MMP,** mucous membrane pemphigoid; **LP,** lichen planus; **HLA,** human leukocyte antigen; **PG,** pemphigoid gestationis; **PNP,** paraneoplastic pemphigus; **eTG/TG3,** epidermal transglutaminase/transglutaminase 3; **EMA,** endomysial antibodies; **SCLE,** subacute cutaneous lupus erythematosus; **SSS,** split skin substrate/salt-split skin; **ME,** monkey esophagus substrate; **NS,** (intact) normal skin substrate; **eTG,** epidermal transglutaminase (transglutaminase 3); **tTG,** tissue transglutaminase (transglutaminase 2); **TG2,** transglutaminase 2 (tissue transglutaminase); **tTG/TG2,** tissue transglutaminase/transglutaminase 2.

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